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Fungal colonization and decomposition of leaves and stems of *Salix arctica* on deglaciated moraines in high-Arctic Canada

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Running title: Decomposition of *Salix* litter in the Arctic

Abstract

Fungal colonization, succession, and decomposition of leaves and stems of *Salix arctica* were studied to estimate the roles of fungi in the decomposition processes in the high Arctic. The samples were collected from five moraines with different periods of development since deglaciation to investigate the effects of ecosystem development on the decomposition processes during the primary succession. The total hyphal lengths and the length of darkly pigmented hyphae increased during decomposition of leaves and stems and were not varied with the moraines. Four fungal morphotaxa were frequently isolated from both leaves and stems. The frequencies of occurrence of two morphotaxa varied with the decay class of leaves and/or stems. The hyphal lengths and the frequencies of occurrence of fungal morphotaxa were positively or negatively correlated with the contents of organic chemical components and nutrients in leaves and stems, suggesting the roles of fungi in chemical changes in the field. Pure culture decomposition tests demonstrated that the fungal morphotaxa were cellulose

decomposers. Our results suggest that fungi took part in the chemical changes in decomposing leaves and stems even under the harsh environment of the high Arctic.

Keywords: Cellulose; Decomposition; Ellesmere Island; Fungi; Succession

1. Introduction

The high-Arctic environment is characterized by low temperature, low moisture, and a short growing season, all of which limit not only net primary productivity and ground cover of plants (Chapin et al., 1992) but also decomposition processes, nutrient availability, and the biological activity in soils that are recently deglaciated and poorly developed (Holding et al., 1974). Studying the roles of fungi in decomposition of plant litter is important for the understanding of high-Arctic ecosystems, as fungi play a central role in the decomposition of lignin and cellulose in plant litter that controls the accumulation and release of essential nutrient for primary production and the buildup of soil organic matter

(Robinson et al., 1995; Osono, 2008). The study of the relationship between fungal succession and decomposition processes is useful to estimate possible roles of fungi in decomposition, but only a few studies have been performed in high-Arctic regions (Robinson et al., 1995, 1996, 1998; Osono et al., 2012), which merit further descriptions of the patterns of fungal colonization, succession, and decomposition decomposer in relation to chemical changes of the litter.

The decomposition of plant litter in high-Arctic regions is expected to exhibit landscape-level variations that relate to the mosaic of habitats differing in microenvironments for biota (Matthews, 1992). Examples include the period of development of the ecosystem since glacier retreat (Jones and Henry, 2003; Mori et al., 2008) and local moisture conditions (Ueno et al., 2009). Such variations affect the development of vegetation and soils, which can in turn lead to the concomitant changes in fungal colonization of plant litter. Primary succession of fungal assemblages following the plant succession after deglaciation has been documented for mycorrhizal fungi (Fujiyoshi et al., 2011) and soil microbes in the arctic (Yoshitake et al., 2006; Schütte et al., 2010). Little

is known, however, how decomposer fungal assemblages change along with the primary succession of vegetation in high-Arctic regions.

The purpose of the present study was to examine fungal colonization and succession in leaves and stems of *Salix arctica*, so as to estimate the roles of fungi in the decomposition in the high Arctic on Ellesmere Island, Nunavut, Canada. Leaves and stems in different stages of decomposition were collected and examined for fungal abundance (as hyphal length) and species composition. Fungi were isolated with a culture-dependent method and identified microscopically and with molecular biological methods to describe fungal assemblages on the litter. Isolates of major fungal species were then tested for their potential to cause chemical changes in *S. arctica* leaves under pure culture conditions to verify roles of these fungal species in decomposition. The study was carried out in five moraines with different periods of development since the last glacial period to test a hypothesis that the decomposer fungal assemblages change along with the primary succession in the high Arctic.

2. Methods

2.1. Study area

The study area is located within the proglacial field of the southern front of Arklio Glacier in the Kreiger Mountains near Oobloyah Bay, Ellesmere Island, Nunavut, Canada. The details of the study area are described in Osono et al. (submitted). The area is rich in well preserved moraines. Arklio Glacier has five glacial moraines, denoted I to V from the closest to the farthest, with different development periods since the Last Glacial (Osono et al. submitted). The order of establishment of these moraines is apparent based on the distance from the present glacier snout.

2.2. Sample collection

Senescent and dead leaves of *S. arctica* were collected from five

moraines in July 2003 (detailed in Osono et al., submitted). Yellowish to reddish senescent leaves attached to current-year shoots were collected from ramets of *S. arctica*. The senescent leaves were regarded as undecomposed materials and denoted as decay class L1. Dead leaves were collected from beneath the prostrating stems of ramets. These dead leaves were divided into two classes according to the estimated age: those that were considered to be dead 1 to 5 years ago (denoted as decay class L2) and those that were considered to be dead 6 to 10 years ago (decay class L3). A total of 20 to 29 leaves were collected qualitatively from each moraine and for each decay class, air-dried *in situ*, and stored in paper bags. These leaves were taken back to the laboratory and used for hyphal length measurement and fungal isolation.

Live and dead stems of *S. arctica* were collected from Moraines II, III, IV, and V in July 2003 (detailed in Osono et al., submitted). Stems were not collected from Moraine I because the standing crop of dead stems of *S. arctica* was very low. Each stem was assigned to five decay classes using visual criteria, including S0 for undecomposed live stems and S1, S2, S3, or S4 for less to more

decomposed dead stems. Six to 16 stems (2.6 to 3.9 mm in diameter, 10 to 15 cm in length) were collected per moraine and decay class, air-dried *in situ*, placed in paper bags, and taken back to the laboratory. Pieces of stems (1 cm in length) were used for hyphal length estimation, and another piece (1 cm) was used for fungal isolation.

2.3. Hyphal length estimation

Hyphal lengths in leaves and stems were estimated using the agar film method of Jones and Mollison (1948) but with several modifications (Osono et al., 2008). One gram of leaf or stem samples was homogenized in a blender at 10,000 rev/min in 49 ml of distilled water for 3 min. The suspension (20 ml) was diluted with 20 ml of molten agar solution (final concentration 1.5%) and mixed at low speed on a magnetic stirring plate. Three agar plates were prepared for each suspension in a haemocytometer (0.1 mm depth), transferred to glass slides, and dried for 24 hours. The films were stained with fluorescent brightener (FB) for

one hour. FB binds to chitin in fungal cell wall (West, 1988) and enables visualization of all hyaline hyphae that are live or ghost (empty). The stained films were mounted between slides and coverslips with one drop of immersion oil (type DF, Cargille Laboratories, Inc., Cedar Grove, NJ, USA) and examined with a Nikon Microphot-SA epifluorescent microscope equipped with a high-intensity mercury light source. A Nikon UV-1A filter cube was used for examination of FB-stained hyphae. Darkly pigmented hyphae that were not stained with FB, were observed by bright field microscopy. Microscope fields were selected randomly and 25 fields were observed for each slide at 1000× magnification. Hyphal lengths were estimated using an eyepiece grid and a grid-intersection method (Olson, 1950). Total hyphal length was calculated as the sum of the lengths of hyaline hyphae stained with FB and darkly pigmented hyphae. Hyphae with clamp connection were classified into Basidiomycota, in spite of the fact that the hyphal length may have been underestimated because the frequency of clamp formation varies between species. Separate litter samples were oven-dried to a constant mass at 40°C to convert to oven-dry

weight

2.4. Fungal isolation

Fungi were isolated from leaves and stems with surface disinfection method (Kinkel and Andrews, 1988) according to Osono et al. (2013). The samples were submerged in 70% ethanol (v/v) for 1 min to wet the surface, surface disinfected for 10 seconds in a solution of 15% hydrogen peroxide (v/v), and then submerged again for 1 min in 70% ethanol. The samples were rinsed with sterile, distilled water, transferred to sterile filter paper in Petri dishes (9cm in diameter), and dried for 24 h to suppress vigorous bacterial growth after plating (Widden and Parkinson, 1973). The samples were then plated on 9-cm Petri dishes containing 2% LCA modified by Miura and Kudo (1970) [glucose 0.1%, KH_2PO_4 0.1%, $MgSO_4 \bullet 7H_2O$ 0.02%, KCl 0.02%, $NaNO_3$ 0.2%, yeast extract 0.02%, and agar 2% (w/v)], two leaves or stems per plate. The plates were incubated at 10°C in darkness and observed at 1, 2, 4 and 8 weeks after the

disinfection. Any fungal hyphae or spores appearing on the plates were subcultured onto fresh LCA plates, incubated, and observed for micromophological characteristics to group into morphotaxa. Identification was made with reference to Ellis (1971, 1976) and Gams (2007). Isolates of major morphotaxa was used for molecular analysis as described below.

The frequency of occurrence of fungal morphotaxa was calculated as a percentage of the number of leaf or stem samples from which a morphotaxa was detected compared with the total number of leaf or stem samples tested for each decay class and each moraine.

2.5. Molecular methods

Genomic DNA was extracted from mycelia that had been cultured on 2.5% malt extract agar [malt extract 2.5%, agar 2% (w/v)] overlaid with a cellophane membrane following the modified CTAB method described by Matsuda and Hijii (1999). Polymerase chain reactions (PCR) were performed using a Quick Taq HS

DyeMix (Toyobo, Osaka, Japan). Each PCR reaction contained a 50 µl mixture (21 µl distilled water, 25 µl master mix, 3 µl ca. 0.5ng/µl template DNA, and 0.5 µl of each primer (final, 0.25 µM)). To PCR amplify the region including the rDNA ITS and 28S rDNA D1-D2 domain, the primer pair ITS1f (Gardes and Bruns, 1993) and LR3 (Vilgalys and Hester, 1990) was used. Each DNA fragment was amplified using a PCR thermal cycler (DNA engine; Bio-Rad, Hercules, CA, USA) using the following thermal cycling schedule. The first cycle consisted of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C for annealing, 1 min at 72°C, and a final cycle of 10 min at 72°C. The reaction mixture was then cooled at 4°C for 5 min. PCR products were purified with a QiAquick PCR Purification Kit (Qiagen, Germany) according to the manufacturer's instructions.

Purified PCR products were sequenced by FASMAC Co., Ltd. (Kanagawa, Japan). Sequencing reactions were performed in a Gene Amp PCR System 9700 (Applied Biosystems, USA) using a BigDye Terminator V3.1 (Applied Biosystems), following the protocols supplied by the manufacturer. The

fluorescent-labeled fragments were purified from the unincorporated terminators using an ethanol precipitation protocol. The samples were resuspended in formamide and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

The sequences determined in this study were deposited in the DNA Data Bank of Japan (DDBJ) (AB751502, AB751503, and AB751504). The rDNA ITS sequences were compared with available rDNA sequences in the GenBank database using BLASTN searches (Altschul et al., 1990). For phylogenetic analysis, MAFFT ver. 6 (Katoh and Toh, 2008) was used for preliminary multiple alignments of nucleotide sequences. Final alignments were manually adjusted using BioEdit (Hall, 1999). Alignment gaps were treated as missing data, and ambiguous positions were excluded from the analysis. The phylogenetic tree was conducted by maximum likelihood (ML) methods (Felsenstein, 1981) with the best-fit nucleotide substitution model based on the lowest Bayesian Information Criterion (BIC) score. To estimate clade support, the bootstrap procedure of Felsenstein (1985) was employed with 1000

replicates. These analyses were carried out using MEGA5 (Tamura et al., 2011).

2.6. Pure culture decomposition test

Nine isolates in 3 major morphotaxa isolated from *S. arctica* leaves were used in a pure culture decomposition test to examine their capacity to decompose leaves. The methods followed those by Osono et al. (2011). Leaves of *S. arctica* in decay classes L1 (senescent) and L2 (decomposed for approximately 1 to 5 years) were used in the test. These leaves were collected from Moraine II in July 2003, air-dried in situ, preserved in paper bags, and taken back to the laboratory. The leaves were then oven-dried to a constant mass at 40°C and preserved in a vinyl bag until the experiment was started.

Leaves (0.6g) were sterilized with ethylene oxide gas at 60°C for 3 h and placed on the surface of Petri dishes (9cm in diameter) containing 20 ml of 2% agar. Inocula for each assessment were cut out of the margin of the previously inoculated Petri dishes on 2% malt extracted agar [malt extract 2%, agar 2%

(w/v)] with a sterile cork borer (6mm in diameter) and placed on the agar adjacent to the leaves, one plug per plate. The plates were incubated for 12 weeks at 10°C in darkness. After incubation, the leaves were retrieved, oven-dried to a constant mass at 40°C, and weighed. Three plates were prepared for each isolate, and three uninoculated plates served as a control. The initial litter was also sterilized, oven-dried to a constant mass at 40°C and weighed to determine the original mass. Mass loss of the leaves was determined as a percentage of the original mass, taking the mass loss of leaves in the uninoculated and incubated control treatment into consideration, and the mean values were calculated for each isolate and each litter type. The leaves from three replicated plates were combined to make one sample and used for analyses of acid unhydrolyzable residue (AUR), total carbohydrates (TCH), carbon (C), and nitrogen (N), according to the method described in Osono et al. (submitted). Mass losses of AUR and TCH were calculated using the same equation as used for the mass loss of leaves.

2.7. Data analysis

The generalized linear model (GLM) was used to evaluate the difference in hyphal length and frequency of fungal morphotaxa in leaves and stems using moraine and decay class as independent variables. GLM was used to evaluate the difference in mass loss of leaves, AUR, TCH, and C and N content in leaves decomposed by fungal isolates under pure culture conditions using fungal morphotaxa and decay class of leaves as independent variables. Tukey's HSD test was performed for multiple comparisons. JMP 6.0 for Macintosh was used to perform these analyses.

3. Results

3.1. Hyphal length

Total hyphal length ranged from 673 to 9470 m/g in leaves and from 537

to 4404 m/g in stems (Table 1) and increased significantly with the decay classes (Table 2). The length of darkly pigmented hyphae ranged from 230 to 2723 m/g in leaves and from 0 to 1128 m/g in stems (Table 1), and it in leaves increased significantly from L1 and L2 to L3 (Table 2). Lengths of darkly pigmented hyphae accounted for 30 ± 4% (mean ± se, n=15) of the total hyphal length in leaves and for 17 ± 2% (n=20) in stems (Table 1). Hyphae with a clamp connection were detected in 5 (33%) out of 15 leaf samples and in 4 (20%) out of 20 stem samples, and these lengths ranged from 24 to 409 m/g and from 16 to 97 m/g, respectively (Table 1). Hyphae with a clamp connection accounted for 1.8 ± 1.0% (n=15) of the total hyphal length in leaves and for 0.9 ± 0.6% (n=20) in stems.

The hyphal lengths were related to chemical composition of leaves and stems (Table 3). Total and darkly pigmented hyphal lengths in leaves were significantly and positively correlated with contents of AUR, N, and Ca and $\delta^{15}N$, and significantly and negatively correlated with extractives (EXT), K, and Mg content and C/N ratio. Total and darkly pigmented hyphal lengths in stems were

significantly and negatively correlated with contents of EXT, N, P, K, and/or Mg.

3.2. Fungal populations

A total of 255 and 136 fungal isolates were obtained from 457 samples of leaves and 223 samples of stems, respectively. Of these isolates, 91% (232 isolates) and 60% (81 isolates), respectively, were sterile and produced no reproductive structures. These sterile mycelia were divided into morphotaxa based on their macro- and micromorphological colony characteristics, and three major morphotaxa (tentatively denoted as sterile mycelia 1Y11, 1L2191, and 2L211) were identified that accounted for 85% (197 isolates) and 75% (61 isolates) of the total number of sterile isolates from leaves and stems, respectively.

DNA analyses confirmed the phylogenetic identity of these morphotaxa as molecular operational taxonomic units. BLAST searches indicated phylogenetic affinities of sterile mycelium 1Y11 to Pleosporales, 1L2191 to

Rhizoctonia, and 2L211 to Dothideomycetes (Table 4). Other isolates that produced reproductive structures, such as conidiophores and spores, during laboratory incubation included *Botrytis cinerea*, *Cladosporium cladosporioides*, *C. herbarum*, *Alternaria* sp., *Aureobasidium pullulans*, *Phialophora* sp., and *Venturia chlorospora*. *Phialophora* sp. was frequently isolated from stems (Table 5).

The frequency of sterile mycelium 1Y11 ranged from 0.0 to 70.0% in leaves and stems and increased significantly from L1 to L2 and then decreased to L3, whereas that in stems decreased from S0 to the other decay classes (Table 5). The frequency of sterile mycelium 1L2191 ranged from 0.0 to 60.0% in leaves and stems, and that in leaves increased significantly from L1 to L2 and L3. The frequency of sterile mycelium 2L211 ranged from 0.0 to 53.3% in leaves and stems. The frequency of *Phialophora* sp. ranged from 0.0 to 38.5% in stems and did not vary significantly with moraine or decay class.

The frequencies of fungi were related to chemical composition of leaves and stems (Table 3). The frequencies of three sterile morphotaxa (1Y11, 1L2919,

and 2L211) in leaves were significantly and positively correlated with contents of AUR, N, and Ca and negatively with contents of EXT, P, K, and/or Mg and C/N ratio. In contrast, those in stems were significantly and positively with contents of EXT, P, K, and Mg and negatively with AUR and TCH.

3.3. Ability of major fungi to decompose S. arctica *leaves*

The chemical contents of undecomposed leaves of *S. arctica* (decay classes L1 and L2) used in pure culture decomposition tests were 298 and 403 mg/g for AUR, 311 and 330 mg/g for TCH, 451 and 460 mg/g for C, and 6.9 and 8.7 mg/g for N, respectively.

Mass loss of leaves ranged from 1.5% to 8.1% (Table 6) and did not vary significantly with morphotaxa or decay class of leaves (GLM, $F=3.3$, d.f.=3, $R^2=0.42$, $P=0.051$). Mass loss of AUR ranged from -10.2% to 0.1% and varied significantly between morphotaxa, but not with decay class ($F=5.5$, d.f.=3, $R^2=0.54$, $P=0.011$). The values of mass loss of AUR was mostly negative (i.e., net

increase of AUR amount) and was significantly lower in leaves decomposed by sterile mycelium 1Y11 than in those decomposed by sterile mycelia 1L2191 and 2L211 ($F=6.2$, d.f.=2, $P=0.012$). Mass loss of TCH ranged from 2.7% to 27.6% and varied significantly with decay class, but not between morphotaxa ($F=4.0$, d.f.=3, $R^2=0.46$, $P=0.031$). Mass loss of TCH in L2 was significantly higher than that in L1 ($F=7.4$, d.f.=1, $P=0.017$).

Carbon content of leaves decomposed by fungal isolates ranged from 464 to 478 mg/g and did not vary significantly between morphotaxa or decay class ($F=0.9$, d.f.=3, $R^2=0.16$, $P=0.49$) (Table 6). Nitrogen content in leaves decomposed by fungal isolates ranged from 8.7 to 10.6 mg/g and varied significantly between morphotaxa, but not with decay class ($F=4.5$, d.f.=3, $R^2=0.49$, $P=0.021$). Nitrogen content in leaves decomposed by sterile mycelium 1Y11 was significantly higher than that in leaves decomposed by sterile mycelium 1L2191 ($F=6.5$, d.f.=2, $P=0.0101$).

4. Discussion

The total hyphal lengths in *S. arctica* leaves and stems were within the range of previous reports for tundra plant litter and soils in the Arctic and Antarctica (i.e., up to 7000 m/g) (Dowding and Widden, 1974; Miller and Laursen, 1974; Robinson et al., 1996). Robinson et al. (1996) reported the fungal hyphal length in litter of *Dryas octopetala* in a high-Arctic polar semi-desert in Svalbard to be 23 m/g, using a membrane filtration method. Osono et al. (2012) reported the total and darkly pigmented hyphal lengths in moss profiles collected from the same study site as the present study to be 104-6310 m/g and 28-3161 m/g, respectively. The net increase in total hyphal length in leaves and stems with decay class is probably attributable to the utilization of organic chemical components by fungal assemblages. The correlation between total hyphal lengths and AUR, EXT, and nutrient contents suggests that these factors potentially limit the fungal growth. The high proportions of darkly pigmented hyphae (up to 59% of the total length) would imply that the production of melanin in fungal cell walls is effective for survival under stress conditions of

low temperature, desiccation, and direct exposure to sunlight on the moraine surfaces (Butler and Day, 1998). The finding that darkly pigmented hyphae in leaves increased with the decay class seems contradictory to the finding that the frequency of occurrence of dark sterile 2L211 was not significantly different between L1, L2, and L3 leaves. This is because the isolation frequency provides little information about hyphal abundance in *S. arctica* leaves.

The finding that most of the isolates were sterile is consistent with previous studies of fungal populations in high-Arctic ecosystems (Widden and Parkinson, 1979; Robinson et al., 1998). Species in the genus *Alternaria*, *Botrytis*, *Cladosporium*, and *Phialophora* isolated from *S. arctica* leaves and stems are common components of fungal assemblages in arctic regions (Cooke and Fournelle, 1960; Widden and Parkinson, 1979; Robinson et al., 1998). Species in *Penicillium*, *Mortierella*, *Chrysosporium*, and *Cylindrocarpon*, dominant fungal species in moss profiles in the study site (Osono et al., 2012), were absent from *S. arctica* leaves and stems, probably because of the difference in the type of substrata (bryophyte versus vascular plant) and in the method of

fungal isolation [the washing method in Osono et al. (2012) versus the surface disinfection method in the present study]. Analyses of rDNA ITS sequences were successful in identifying the major morphotaxa 1Y11, 1L2191, and 2L211 to order (Pleosporales), genus (*Rhizoctonia*), and class (Dothideomycets) levels, respectively. No record is available in GenBank (as of December 2012) for rDNA ITS sequences closely related to 1Y11. A species in *Rhizoctonia*, most closely related to 1L2191, was reported as a common non-mycorrhizal endophyte of *Salix* trees in boreal and alpine regions in Norway (Dhillion, 1994). A species of *Rhizoctonia* is also known as a pathogen of carrot in cold storage at 1-3˚C (Adams and Kropp, 1996). *Xenostigmina zilleri*, most closely related to 2L211, was isolated from *Acer* leaves in British Columbia, Canada (Crous et al., 2009), but its role as a decomposer is unknown.

The three morphotaxa 1Y11, 1L2191, and 2L211, and *Phialophora* sp. were generally and consistently dominant on *S. arctica* leaves and/or stems, indicating that the species turnover of fungal assemblages was not obvious during the decomposition or the ecosystem development. The extremely adverse

environment may limit the coexistence of multiple fungal species and may account for the dominance and ubiquity of the limited number of fungal species in the study site. However, the frequency of 1Y11 and 1L2191 varied with the decay class of leaves and/or stems and was correlated with the organic chemical and nutrient contents, suggesting that chemical changes during the decomposition potentially affected the growth and occurrence of fungal morphotaxa.

The selective loss of TCH during the decomposition of *S. arctica* leaves by three morphotaxa under pure culture indicates that they are cellulolytic and suggests that these fungi are responsible for the loss of TCH in leaves from the L1 to L2 and L3 decay classes. The negative values of mass loss of AUR and the increase of N content in the decomposed leaves indicate the net increase of recalcitrant compounds and immobilization of N in the leaves during the fungal cellulolysis. The increase in recalcitrant compounds in association with N retention has previously been reported to occur in the high-Arctic (Osono et al., 2012), in temperate forests (Osono et al., 2006a), and in the laboratory (Osono et

al., 2006b) and is explained by the activity of fungi that produce recalcitrant nitrogenous compounds (Berg, 1986). Our data also showed that the net increase of AUR and the increase of N content occurred more in L1 leaves than in L2 leaves during the pure culture decomposition. These results are consistent with the field observation that such changes in AUR and N were more evident in decomposing leaves from L1 to L2 than in those from L2 to L3. The results of laboratory experiments do not necessarily represent the activity of fungal morphotaxa in the field, but our results support the idea that the ingrowth, colonization, and activity of fungi in *S. arctica* accounted for the chemical changes and that fungi took part in the decomposition processes, even in the harsh environment of the high Arctic.

Our results, together with the results of chemical analyses of leaves and stems (Osono et al. unpublished), have shown the patterns of changes in chemical and fungal properties of leaf and stems litter of *S. arctica* in the study site and suggested that fungi play major roles in the transformation of litter. These do not exclude the possible importance of unobserved biotic components,

such as bacteria and soil animals or the overwhelming effects of abiotic conditions in arctic soils. Future research directions include a long-term decomposition experiment using litterbags to follow quantitatively changes in chemical properties and biological assemblages during decomposition and to verify the findings of the present study.

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References

Adams, G.C., Kropp, B.R., 1996. *Athelia arachnoidea*, the sexual state of *Rhizoctonia carotae*, a pathogen of carrot in cold storage. Mycologia 88, 459-472.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403-410.

Berg, B., 1986. Nutrient release from litter and humus in coniferous forest soils - a mini review. Scand. J. For. Res. 1, 359-369.

Butler, M.J., Day, A.W., 1998. Fungal melanins: a review. Can. J. Microbiol. 44, 1115-1136.

Chapin, III, F.S., Jefferies, R.L., Reynolds, J.F., Shaver, G.R., Svoboda, J., 1992.

Arctic Ecosystems in a Changing Climate. An Ecophysiological Perspective. Academic Press, London.

Cooke, W.B., Fournelle, H.T., 1960. Some soil fungi from an Alaskan tundra area. Arctic 13, 266-270.

Crous, P.W., Braun, U., Wingfield, M.J., Wood, A.R., Shin, H.D., Summerell, B.A., Alfenas, A.C., Cumagun, C.J., Groenewald, J.Z., 2009. Phylogeny and taxonomy of obscure genera of microfungi. Persoonia 22, 139-161.

Dhillion, S.S., 1994. Ectomycorrhizae, arbuscular mycorrhizae, and *Rhizoctonia* sp. of alpine and boreal *Salix* spp. in Norway. Arct. Alp. Res. 26, 304-307.

Dowding, P., Widden, P., 1974. Some relationships between fungi and their environment in tundra regions, in: Holding, A.J., Heal, O.W., MacLean, Jr., S.F., Flangan, P.W. (Eds) Soil Organisms and Decomposition in Tundra. Tundra Biome Steering Committee, University of Alaska, AL, pp 123-150.

Ellis, M.B., 1971. Dematiaceous hyphomycetes. CAB International, Oxon, UK.

Ellis, M.B., 1976. More dematiaceous hyphomycetes. CAB International, Oxon, UK.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368-376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783-791.

Fujiyoshi, M., Yoshitake, S., Watanabe, K., Murota, K., Tsuchiya, Y., Uchida, M., Nakatsubo, T., 2011. Successional changes in ectomycorrhizal fungi associated with the polar willow *Salix polaris* in a deglaciated area in the High Arctic, Svalbard. Polar Biol. 34, 667-673.

Gams, W., 2007. Compendium of soil fungi, second edition. IHW-Verlag & Verlagsbuchhandlung, Germany.

Gardes, M., Bruns, T.D., 1993. ITS primer with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rust. Mol. Ecol. 21, 113-118.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor

and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95-98.

Holding, A.J., Heal, O.W., MacLean, S.F., Flangan, Jr., P.W., 1974. Soil Organisms and Decomposition in Tundra. Tundra Biome Steering Committee, University of Alaska, AL.

Jones, P.C.T., Mollison, J.E., 1948. A technique for the quantitative estimation of soil microorganisms. J. Gen. Microbiol. 2, 54-69.

Jones, G.A., Henry, G.H.R., 2003. Primary plant succession on recently deglaciated terrain in the Canadian High Arctic. J. Biogeogr. 30, 277-296.

Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9, 276-285.

Kinkel, L.L., Andrews, J.H., 1988. Disinfection of living leaves by hydrogen peroxide. Trans. Br. Mycol. Soc. 91, 523-528.

Matsuda, Y., Hijii, N., 1999. Characterization and identification of *Strobilomyces confusus* ectomycorrhizas on momi fir by RFLP analysis

of the PCR-amplified ITS region of the rDNA. J. For. Res. 4, 145-150.

Matthews, J.A., 1992. The Ecology of Recently-Deglaciated Terrain. A geoecological approach to glacier forelands and primary succession. Cambridge University Press, Cambridge.

Miller, Jr., O.K., Laursen, G.A., 1974. Belowground fungal biomass on US tundra biome sites at Barrow, Alaska, in: Holding, A.J., Heal, O.W., MacLean, Jr., S.F., Flangan, P.W. (Eds) Soil Organisms and Decomposition in Tundra. Tundra Biome Steering Committee, University of Alaska, AL, pp 151-158.

Miura, K., Kudo, M., 1970. An agar-medium for aquatic hyphomycetes. Trans. Mycol. Soc. Japan 11, 116-118.

Mori, A., Osono, T., Uchida, M., Kanda, H., 2008. Changes in the structure and heterogeneity of vegetation and microsite environments with the chronosequence of primary succession on a glacier foreland in Ellesmere Island, high arctic Canada. Ecol. Res. 23, 363-370.

Olson, F.C.W., 1950. Quantitative estimates of filamentous algae. T. Am. Microsc.

Soc. 69, 272-279.

Osono, T., 2005. Colonization and succession of fungi during decomposition of *Swida controversa* leaf litter. Mycologia 97, 589-597.

Osono, T., 2008. Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecol. Res. 22, 955-974.

Osono, T., Hobara, S., Koba, K., Kameda, K., Takeda, H., 2006a. Immobilization of avian excreta-derived nutrients and reduced lignin decomposition in needle and twig litter in a temperate coniferous forest. Soil Biol. Biochem. 38, 517-525.

Osono, T., Hobara, S., Koba, K., Kameda, K., 2006b. Reduction of fungal growth and lignin decomposition in needle litter by avian excreta. Soil Biol. Biochem. 38, 1623-1630.

Osono, T., Ishii, Y., Hirose, D., 2008. Fungal colonization and decomposition of *Castanopsis sieboldii* leaf litter in a subtropical forest. Ecol. Res. 23, 909-917.

Osono, T., Hobara, S., Hishinuma, T., Azuma J.I., 2011. Selective lignin

decomposition and nitrogen mineralization in forest litter colonized by *Clitocybe* sp. Eur. J. Soil Biol. 47, 114-121.

Osono, T., Ueno, T., Uchida, M., Kanda, H., 2012. Abundance and diversity of fungi in relation to chemical changes in arctic moss profiles. Polar Sci. 6, 121-131.

Osono, T., Tateno, O., Masuya, H., 2013. Diversity and ubiquity of xylariaceous endophytes in live and dead leaves of temperate forest trees. Mycoscience 54, 54-61.

Osono, T., Uchida, M., Kanda, H. Chemical changes in decomposing leaves and stems of *Salix arctica* on deglaciated moraines in high-Arctic Canada. Polar Sci. submitted.

Robinson, C.H., Wookey, P.A., Parsons, A.N., Potter, J.A., Callaghan, T.V., Lee, J.A., Press, M.C., Welker, J.M., 1995. Responses of plant litter decomposition and nitrogen mineralization to simulated environmental changes in a high arctic polar semi-desert and a subarctic dwarf shrub heath. Oikos 74, 503-512.

Robinson, C.H., Borisova, O.B., Callaghan, T.V., Lee, J.A., 1996. Fungal hyphal length in litter of *Dryas octopetala* in a high-Arctic polar semi-desert, Svalbard. Polar Biol. 16, 71-74.

Robinson, C.H., Fisher, P.J., Sutton, B.C., 1998. Fungal biodiversity in dead leaves of fertilized plants of *Dryas octopetala* from a high arctic site. Mycol. Res. 102, 573-576.

Schütte, U.M.E., Abdo, Z., Foster, J., Ravel, J., Bunge, J., Solheim, B., Forney, L.J., 2010. Bacterial diversity in a glacier foreland of the high Arctic. Mol. Ecol. 19, 54-66.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731-2739.

Ueno, T., Osono, T., Kanda, H., 2009. Inter- and intraspecific variations of the chemical properties of high-Arctic mosses along water-regime gradients. Polar Sci. 3, 134-138.

Vilgalys, R., Hester, M., 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172, 4238-4246.

West, A.W., 1988. Specimen preparation, stain type, and extraction and observation procedures as factors in the estimation of soil mycelial lengths and volumes by light microscopy. Biol. Fertil. Soils 7, 88-94.

Widden, P., Parkinson, D., 1973. Fungi from Canadian coniferous forest soils. Can. J. Bot. 51, 2275-2290.

Widden, P., Parkinson, D., 1979. Populations of fungi in a high arctic ecosystems. Can. J. Bot. 57, 2408-2417.

Yoshitake, S., Uchida, M., Nakatsubo, T., Kanda, H., 2006. Characterization of soil microflora on a successional glacier foreland in the high Arctic on Ellesmere Island, Nunavut, Canada using phospholipid fatty acid analysis. Polar Biosci. 19, 73-84.

T. Osono et al. Table 1

Table 1. Length (m/g dry material) of total and darkly pigmented hyphae and the proportion of darkly pigmented hyphal length relative to the total hyphal length (%) in *Salix arctica* leaves and stems. nd, not determined.

		Leaves			Stems				
	Moraine	L1	L2	L3	S0	S1	S2	S3	S4
Total	I	2168	4112	5199	nd	nd	nd	nd	nd
	II	1123	4713	8400	722	1261	1430	1809	2425
	III	831	2909	5563	537	2136	2784	2896	4404
	IV	673	5858	9470	631	971	2106	1551	3406
	V	955	3974	5074	1218	2217	2249	2591	2064
Darkly	I	474	1103	898	nd	nd	nd	nd	nd
pigmented	II	618	691	2723	48	168	182	258	320
	III	396	461	2494	0	356	344	551	1128
	IV	396	1354	2247	57	170	417	325	649
	V	230	685	1174	554	703	759	387	0
% darkly	I	22	27	17	nd	nd	nd	nd	nd
pigmented	II	55	15	32	7	13	13	14	13
hyphae	III	48	16	45	0	17	12	19	26
	IV	59	23	24	9	18	20	21	19
	V	24	17	23	45	32	34	15	0

T. Osono et al. Table 2

Table 2. Results of generalized linear models to evaluate the difference in hyphal length and frequency of fungal morphotaxa using moraine and decay class as independent variables. n=15 and n=20 for leaves and stems, respectively. *** P<0.001, ** P<0.01, * P<0.05, ns non significant.

	Leaves						Stems					
	Model			Moraine		Decay class	Model			Moraine		Decay class
	R^2	F		F		F	R^2	F		F		F
Hyphal length												
Total	0.88	9.9	**	1.8	ns	26.1 ***	0.79	6.3	**	3.0	ns	8.7 **
Darklyl-pigmented	0.75	4.1	*	0.9	ns	10.4 **	0.35	0.9	ns	1.1	ns	0.8 ns
% darklyl-pigmented	0.62	2.2	ns	1.2	ns	4.3 ns	0.26	0.6	ns	1.2	ns	0.2 ns
Frequency of fungi												
1Y11	0.91	14.2	***	2.0	ns	38.6 ***	0.60	2.6	ns	1.5	ns	3.5 *
1L2191	0.81	5.8	*	2.9	ns	11.6 **	0.55	2.1	ns	2.7	ns	1.6 ns
2L211	0.73	3.7	*	3.3	ns	4.3 ns	0.43	1.3	ns	0.5	ns	1.9 ns
Phialophora sp.	0.43	1.0	ns	1.0	ns	1.0 ns	0.45	1.4	ns	1.7	ns	1.2 ns

T. Osono et al. Table 3

Table 3. Correlation between fungal assemblages and chemical properties. Pearson's correlation coefficients were calculated for linear relationship between hyphal lengths or frequencies of major fungal morphotaxa and contents of organic chemical components and nutrients. Data of chemical properties are after Osono et al. submitted. Chemical properties with significant correlation coefficients ($P<0.05$) are indicated. nd, no chemical properties gave significant correlation coefficients. AUR, acid unhydrolyzable residue; TCH, total carbohydrates; EXT, extractives; $\delta^{15}N$, natural abundance of ^{15}N.

	Leaves (n=15)		Stems (n=20)	
	Positive	Negative	Positive	Negative
Hyphal length				
Total	AUR, N, Ca, $\delta^{15}N$	EXT, K, Mg, C/N ratio	nd	EXT, P, K, Mg
Darkly pigmented	AUR, N	EXT, K, Mg, C/N ratio	nd	N, P
% darkly pigmented hyphae	EXT	Ca	$\delta^{15}N$	nd
Frequency of major morphotaxa				
1Y11	AUR, Ca	EXT, K, Mg	EXT, P, K, Mg	AUR, TCH
1L2191	AUR	EXT, P, K, Mg	nd	nd
2L211	N	C/N ratio	EXT, P	AUR
Phialophora sp.	P, K	nd	nd	nd

T. Osono et al. Table 4

Table 4. DNA sequence analysis results for the rDNA-internal transcribed spacer (ITS) region based on BLAST searches for three major morphotaxa isolated from *Salix arctica* leaves and stems.

Morphotaxon	DDBJ accession number	Closest match at Genbank (Accession number)	Query coverage %	Sequence similarity %
1Y11	AB751503	*Alternaria brassicicola* (AY154707)	100	87
1L2191	AB751502	*Rhizoctonia* sp. (AJ419931)	94	97
2L211	AB751504	*Xenostigmina zilleri* (FJ839639)	97	96

T. Osono et al. Table 5

Table 5. Frequency of occurrence (%) of major fungal morphotaxa in *Salix arctica* leaves and stems. nd, not determined.

		Leaves			Stems				
Morphotaxon	Moraine	L1	L2	L3	S0	S1	S2	S3	S4
1Y11	I	14.3	70.0	28.6	nd	nd	nd	nd	nd
	II	0.0	43.5	12.0	0.0	0.0	0.0	0.0	0.0
	III	0.0	68.2	29.2	31.3	33.3	0.0	0.0	0.0
	IV	0.0	62.5	33.3	25.0	0.0	0.0	0.0	0.0
	V	0.0	35.0	33.3	60.0	0.0	7.7	7.7	0.0
1L2191	I	0.0	0.0	9.5	nd	nd	nd	nd	nd
	II	0.0	30.4	24.0	0.0	0.0	0.0	9.1	0.0
	III	0.0	27.3	50.0	6.3	0.0	0.0	13.3	0.0
	IV	0.0	37.5	54.2	33.3	16.7	10.0	12.5	0.0
	V	0.0	60.0	52.4	13.3	28.6	0.0	7.7	9.1
2L211	I	0.0	0.0	4.8	nd	nd	nd	nd	nd
	II	0.0	30.4	32.0	53.3	0.0	0.0	9.1	9.1
	III	0.0	0.0	12.5	31.3	0.0	5.9	0.0	11.1
	IV	0.0	0.0	16.7	8.3	16.7	0.0	0.0	11.1
	V	0.0	0.0	4.8	0.0	0.0	7.7	7.7	9.1
Phialophora sp.	I	4.8	0.0	0.0	nd	nd	nd	nd	nd
	II	0.0	0.0	0.0	0.0	16.7	38.5	9.1	36.4
	III	0.0	0.0	0.0	12.5	33.3	23.5	13.3	0.0
	IV	0.0	0.0	0.0	0.0	0.0	20.0	0.0	11.1
	V	0.0	0.0	0.0	0.0	0.0	0.0	15.4	18.2

T. Osono et al. Table 6

Table 6. Mass loss (% initial mass) of leaves, acid unhydrolyzable residue (AUR), and total carbohydrates (TCH) and contents (mg/g) of carbon (C) and nitrogen (N) in *Salix arctica* leaves (decay class L1 and L2) decomposed *in vitro* for 12 weeks at 10˚C by three fungal morphotaxa. Values indicate means ± standard errors of three strains for each morphotaxa. na not applicable.

Morphotaxon	Decay class	Mass loss			Content	
		Leaves	AUR	TCH	C	N
1Y11	L1	1.5 ± 1.7	-10.2 ± 2.4	2.7 ± 3.3	464 ± 2	10.5 ± 0.4
	L2	4.8 ± 1.4	-6.7 ± 1.2	14.7 ± 3.4	471 ± 2	10.6 ± 0.3
1L2191	L1	2.9 ± 0.5	-4.8 ± 2.6	3.8 ± 5.4	466 ± 2	8.7 ± 0.2
	L2	6.2 ± 2.3	-0.9 ± 0.4	19.9 ± 5.6	471 ± 1	9.3 ± 0.5
2L211	L1	6.6 ± 2.7	-2.8 ± 3.5	14.8 ± 11.6	478 ± 8	9.8 ± 0.7
	L2	8.1 ± 1.6	0.1 ± 2.1	27.6 ± 6.8	470 ± 5	9.7 ± 0.5
Undecomposed material	L1	na	na	na	451	6.9
	L2	na	na	na	460	8.7

Electronic Supplementary Material

Fungal colonization and decomposition of leaves and stems of *Salix arctica* on deglaciated moraines in high-Arctic Canada

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S1. Study area

The study area (80°50-52'N, 82°49-51'W, WGS84) is located within the proglacial field of the southern front of Arklio Glacier in the Kreiger Mountains near Oobloyah Bay, Ellesmere Island, Nunavut, Canada. The details of the study area are described in Mori et al. (2008). The geological frame of the study area is mainly built up of Younger Paleozoic and Mesozoic sedimentary rocks, dominated by sandstone, siltstone and shale, of the Sverdrup Basin (Okitsu et al., 2004). Weathering of bedrocks and soil development was generally poor. This was probably due to low temperature and relatively short periods (estimated 25000 years; Hasegawa et al., 2004) since the recession of the glacier. No climatic data are available in the study area. However, the climate as represented by the weather station at Eureka (80°00'N, 85°56'W), located 130 km south of the study area, is extremely harsh. Annual mean temperature is -19.7°C and monthly mean temperature of the warmest (July) and coldest (February) month is 3.3°C and -38.0°C, respectively. Annual precipitation is 64 mm (Atmospheric Environmental Service, 1982; after Kojima, 1994).

The area is rich in well preserved moraines. Arklio Glacier has five glacial moraines, denoted I to V from the closest to the farthest, with different development periods since the Last Glacial Maximum (Table S1). The order of establishment of these moraines is apparent based on the distance from the present glacier snout. Their age has been estimated to be 300 years for Moraine I to at least 25000 years since deglaciation for Moraine V (Hasegawa et al., 2004). Moraine I is covered thoroughly with fresh, sharp-edged rocks and the colonization of vascular plants, bryophytes, and lichens is very limited with plant coverage less than 1% of the surface. The plant covers increase from Moraine II to V that are dominated by vascular plants, such as *Salix arctica*, *Dryas integrifolia*, and *Cassiope tetragona* (Okitsu et al., 2004; Mori et al., 2006, 2008), and bryophytes, such as

Racomitrium laguninosum and *Hylocomium splendens* (Ueno et al., 2009; Osono et al., 2012).

Table S1. Locations, development periods, and estimated ages of five terminal moraines of Arklio glacier.

Moraine	Distance from glacial snout (m)	Elevation (m)	Development period	Estimated age (years ago)
I	0-250	280-320	Little Ice Age	250-400
II	250-600	230-260	Neoglaciation	2400-3300
III	600-1150	210-230	Early Holocene	8000
IV	1150-2650	130-200	Late Glacial	12000-15000
V	2650-3000	120-130	Full Glacial	25000-35000

The periods of development of the moraines were estimated according to geomorphological observations, relative dating by weathering rind thickness in sandstone gravels and lichenometry using *Rhizocarpon geographicum* s.l., and ^{14}C age dating of buried organic deposits (Hasegawa et al., 2004).

References

Hasegawa, H., Sawaguchi, S.I., Amaizawa, A., 2004. Later Quaternary glaciation in the Oobloyah Bay area, N.W. Ellesmere Island, Canada. Sundai Historical Review 123, 1-28.

Kojima, S., 1994. Relationships of vegetation, earth hummocks, and topography in the high arctic environment of Canada. Proc. NIPR Symp. Polar Biol. 7, 256-269.

Mori, A., Osono, T., Iwasaki, S., Uchida, M., Kanda, H., 2006. Initial recruitment and establishment of vascular plants in relation to topographical variation in microsite conditions on a receltly-deglaciated moraine on Ellesmere Island, high arctic Canada. Polar Biosci. 19, 85-95.

Mori, A., Osono, T., Uchida, M., Kanda, H., 2008. Changes in the structure and heterogeneity of vegetation and microsite environments with the chronosequence of primary succession on a glacier foreland in Ellesmere Island, high arctic Canada. Ecol. Res. 23, 363-370.

Okitsu, S., Sawaguchi, S., Hasegawa, H., Kanda, H., 2004. Vegetation development on the

glacier moraines in Oobloyah Valley, Ellesmere Island, high arctic Canada. Polar Biosci. 17, 83-94.

Osono, T., Ueno, T., Uchida, M., Kanda, H., 2012. Abundance and diversity of fungi in relation to chemical changes in arctic moss profiles. Polar Sci. 6, 121-131.

Ueno, T., Osono, T., Kanda, H., 2009. Inter- and intraspecific variations of the chemical properties of high-Arctic mosses along water-regime gradients. Polar Sci. 3, 134-138.

S2. Sample collection

Senescent and dead leaves of *Salix arctica* were collected from five moraines in July 2003. Five ramets of S. arctica were chosen on each moraine, and yellowish to reddish senescent leaves attached to current-year shoots were collected. The senescent leaves were regarded as undecomposed materials and denoted as decay class L1 (Table S2). Dead leaves were collected from beneath the prostrating stems of ramets, as long as the ramets were sheltered from the wind in depressions or between the boulders and when we confirmed that the leaves were derived from the stems right above them, according to the position of leaf trace and bud scar on stems and the direction of leaf petiole. Time of leaf death was estimated by counting the number of annual bud scars remaining on the stems just above the dead leaves. These dead leaves were divided into two classes according to age: those that were considered to be dead 1 to 5 years ago (denoted as decay class L2) and those that were considered to be dead 6 to 10 years ago (decay class L3) (Table S2). These leaves were pressed between board papers, dried at 40°C for one week, and used for chemical analyses.

Live and dead stems of *S. arctica* were collected from Moraines II, III, IV, and V in July 2003. Stems were not collected from Moraine I because the standing crop of dead stems of S. arctica was very low (0.1 ± 0.0 g/m2, mean ± standard errors, n=20) compared with Moraines II (12.9 ± 2.9 g/m2), III (2.4 ± 1.0 g/m2), IV (4.3 ± 1.2 g/m2), and V (7.5 ± 1.4 g/m2). Each stem was assigned to five decay classes using visual criteria (Table S2). Dead stems were classified into decay class S1, S2, S3, or S4 according to the presence/absence of dead leaves and/or flowers, the color of bark, and the degree of loss of bark. That is, stems in decay class S1 represented an early stage of decomposition, whereas those in decay class S4 represented an advanced stage (Table S2). Live stems were regarded as undecomposed materials and denoted as decay class S0 (Table S2). Six to 16 stems (2.6 to 3.9 mm in diameter, 10 to 15 cm in length) were collected per moraine and decay class,

air-dried, placed in paper bags, and taken back to the laboratory. Pieces of stems (2 cm in length) were used for hyphal length estimation and fungal isolation, and the remaining stem (8 to 13 cm) was dried at 40°C for one week and used for chemical analyses.

Table S2. Decay class of leaves and stems of *Salix arctica*.

Decay class	Description
Leaves	
L1	Senescent, yellowish leaves attached on shoots
L2	Brown leaves detached and experienced decomposition for 1 to 5 years
L3	Brown to whitish leaves detached and experienced decomposition for 6 to 10 years
Stems	
S0	Live, bark brown and intact
S1	Dead, bark brown and intact, dead leaves or flowers attached
S2	Dead, bark white and partly cracked, dead leaves or flowers detached
S3	Dead, bark white and detached less than 50% of total stem surface
S4	Dead, bark white and detached more than or equal to 50% of total stem surface

S3. Chemical analyses

Leaves and stems were ground in a laboratory mill to produce particles that would pass through a 0.5-mm screen, combined in a single sample of leaves or stems per moraine and per decay class, and used for chemical analyses (Table S3). Acid-unhydrolyzable residue (AUR, also known as the acid-insoluble residue or 'Klason lignin' fraction) and extractive (EXT) contents were measured by sulfuric acid digestion and alcohol-benzene extraction, respectively (King and Heath, 1967). Acid-unhydrolyzable residue contains not only true lignin of plant origin but also condensed tannin, phenolic compounds, carboxylic compounds, and alkyl compounds such as cutin (Preston et al., 1997). Total carbohydrate (TCH) content was measured by a phenol-sulfuric acid method (Dubois et al., 1956). The methods of these proximate analyses are described in detail in Osono et al. (2008).

Total C and total N contents were measured by automatic gas chromatography (NC analyzer SUMIGRAPH NC-900, Sumitomo Chemical Co., Osaka, Japan). After acid wet oxidation in HNO_3 + HClO, an ascorbic acid method was performed for P (Olsen and

Sommers, 1982); flame photometry was performed for K, and atomic absorption was performed for Ca and Mg (atomic absorption spectrophotometer 170-30S; Hitachi, Tokyo, Japan). Details of the methods were described by Osono and Takeda (2004, 2005) and Osono et al. (2006). The contents were expressed in mg/g ash-free dry material.

Nitrogen stable isotope ratio of leaves and stems was determined on a mass spectrometer (Finnigan Mat Delta S, Bremen, Germany). Isotopic composition is expressed in per mil (‰) deviation from the atmospheric N2, which is defined by the following equation: $\delta^{15}N = [(^{15}N/^{14}N \text{ sample}/^{15}N/^{14}N \text{ standard}) - 1] \times 1000$. α-D,L-alanine was used as the internal standard ($\delta^{15}N = -1.7$‰). The standard deviation based on analyses of replicated samples was <0.16‰.

Table S3. Contents (mg/g) of organic chemical components, carbon, nutrients, C/N ratio, and $\delta^{15}N$ (‰) in *Salix arctica* leaves and stems. nd, not determined. AUR, acid unhydrolyzable residue; TCH, total carbohydrates; EXT, extractives.

		Leaves			Stems				
Component	Moraine	L1	L2	L3	S0	S1	S2	S3	S4
AUR	I	302	402	381	nd	nd	nd	nd	nd
	II	321	435	426	303	442	423	428	354
	III	337	449	430	302	423	414	421	368
	IV	341	429	429	310	399	401	397	355
	V	352	426	432	310	436	408	372	322
TCH	I	270	309	307	nd	nd	nd	nd	nd
	II	321	293	280	410	395	414	437	503
	III	265	298	268	346	353	361	397	428
	IV	246	301	287	374	363	396	449	459
	V	212	256	263	348	377	375	425	492
EXT	I	119	65	52	nd	nd	nd	nd	nd
	II	139	68	59	218	73	61	61	78
	III	165	53	51	211	95	90	61	55
	IV	113	52	38	181	83	50	53	73
	V	131	55	36	206	58	80	67	73
C	I	432	446	418	nd	nd	nd	nd	nd

	II	461	468	454	515	514	515	517	502
	III	469	465	447	513	522	526	519	499
	IV	463	462	459	508	507	508	507	501
	V	466	458	452	502	504	512	501	493
N	I	7.5	9.2	11.2	nd	nd	nd	nd	nd
	II	7.1	9.5	11.7	5.4	5.3	4.3	4.4	4.3
	III	7.5	8.1	10.7	5.4	4.5	4.6	4.3	4.1
	IV	8.9	8.2	11.1	4.7	3.5	3.2	3.4	3.6
	V	10.3	8.2	10.6	5.2	2.9	3.6	3.8	4.5
P	I	0.68	0.55	0.33	nd	nd	nd	nd	nd
	II	0.18	0.21	0.15	0.47	0.35	0.22	0.19	0.18
	III	0.14	0.09	0.13	0.64	0.11	0.16	0.10	0.18
	IV	0.12	0.09	0.13	0.50	0.10	0.15	0.15	0.15
	V	0.27	0.09	0.11	0.56	0.15	0.17	0.19	0.11
K	I	20.2	3.3	2.5	nd	nd	nd	nd	nd
	II	9.0	2.6	1.4	2.6	1.9	0.9	0.6	0.3
	III	8.2	1.2	1.4	3.0	1.9	1.3	0.5	0.4
	IV	9.3	2.4	1.2	2.7	1.7	0.6	0.4	0.5
	V	10.1	2.6	1.5	2.7	1.6	0.9	0.5	0.4
Ca	I	21.5	26.2	27.4	nd	nd	nd	nd	nd
	II	14.3	16.0	19.0	5.5	7.7	5.9	5.9	3.5

			9	7					
			19.	24.					
	III	13.7	9	1	7.3	6.2	7.1	5.4	4.5
			21.	23.					
	IV	15.1	7	3	6.0	8.5	7.8	6.4	6.6
			24.	25.					
	V	14.4	0	3	6.2	8.6	5.9	5.4	6.7
			1.3	1.7					
Mg	I	3.08	8	6	nd	nd	nd	nd	nd
			1.7	1.2		0.5	0.3	0.4	0.2
	II	2.76	2	1	0.62	8	8	6	8
			1.4	1.1		0.8	0.5	0.3	0.2
	III	2.67	3	4	0.69	1	6	7	7
			1.6	0.9		0.4	0.2	0.2	0.2
	IV	2.18	7	6	0.52	2	8	0	7
			2.1	1.1		0.6	0.4	0.3	0.3
	V	2.49	5	7	0.65	6	3	5	5
C/N ratio	I	57	49	37	nd	nd	nd	nd	nd
	II	65	49	39	95	96	121	119	118
	III	62	58	42	96	115	113	121	121
	IV	52	56	41	109	146	159	151	138
	V	45	56	42	97	173	143	131	110
$\delta^{15}N$	I	-4.7	-5.4	-4.3	nd	nd	nd	nd	nd
	II	-5.7	-5.7	-4.8	-5.4	-4.3	-4.8	-5.1	-4.8
	III	-6.6	-5.4	-5.2	-5.7	-5.5	-5.4	-5.3	-5.1
	IV	-5.2	-4.6	-4.8	-5.7	-5.7	-5.2	-4.8	-5.2
	V	-5.8	-5.7	-5.1	-3.8	-5.1	-4.1	-5.0	-4.8

References

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350-356.

King, H.G.C., Heath, G.W., 1967. The chemical analysis of small samples of leaf material

and the relationship between the disappearance and composition of leaves. Pedobiologia 7, 192-197.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus, in: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.) Methods of Soil Analysis, Part 2. ASA and SSSA, Madison, WI, pp 403-430.

Osono, T., Takeda, H., 2004. Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species in a cool temperate forest. Ecol. Res. 19, 593-602.

Osono, T., Takeda, H., 2005. Decomposition of organic chemical components in relation to nitrogen dynamics in leaf litter of 14 tree species in a cool temperate forest. Ecol. Res. 20, 41-49.

Osono, T., Mori, A., Uchida, M., Kanda, H., 2006. Chemical property of live and dead leaves of tundra plant species in Oobloyah Valley, Ellesmere Island, high arctic Canada. Mem. Natl. Inst. Polar Res., Spec. Issue 59, 144-155.

Osono, T., Ishii, Y., Hirose, D., 2008. Fungal colonization and decomposition of *Castanopsis sieboldii* leaves in a subtropical forest. Ecol. Res. 23, 909-917.

Preston, C.M., Trofymow, J.A., Sayer, B.G., Niu, J., 1997. ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies. Can. J. Bot. 75, 1601-1613.